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Nociceptin inhibits capsaicin-induced bronchoconstriction in isolated guinea pig lung

Michel R. Corboz [a,*], Maria A. Rivelli [a], Robert W. Egan [a], Deen Tulshian [b], Julius Matasi [b], Ahmad B. Fawzi [c], Lawrence Benbow [c], April Smith-Torhan [c], Hongtao Zhang [c], John A. Hey [a]

[a] *Allergy, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*
[b] *Chemistry, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*
[c] *CNS Biology, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA*

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Abstract

The isolated perfused guinea pig lung was used to investigate the effect of nociceptin against bronchoconstriction elicited by endogenous and exogenous tachykinins. The opioid receptor-like 1 (ORL1) receptor agonist, nociceptin/orphanin FQ (0.001–1 μM) produced a dose-related inhibition of the capsaicin-induced bronchoconstriction (10^{-5}–10^{3} μg) in isolated guinea pig lung ($P < 0.05$), a response mediated by the release of endogenous tachykinins from lung sensory nerves. The new ORL1 receptor antagonist 1-[(3*R*,4*R*)-1-Cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397) (0.3 μM) significantly blocked the inhibitory effect of nociceptin/orphanin FQ (0.01 μM) on capsaicin-induced bronchoconstriction, whereas the non-selective opioid receptor antagonist naloxone (1 μM) had no effect. Nociceptin/orphanin FQ (1 μM) did not affect the bronchoconstriction induced exogenously by the tachykinin NK2 receptor agonist neurokinin A. In conclusion, the present data provide evidence that nociceptin inhibits capsaicin-evoked tachykinin release from sensory nerve terminals in guinea pig lung by a prejunctional mechanism. This inhibitory action occurs independently from activation of opioid receptors. The present study also indicates that J-113397 is a potent ORL1 receptor antagonist.

Keywords: Isolated lung, guinea pig; ORL1 receptors; Nociceptin/orphanin FQ; ORL1 receptor antagonist

1. Introduction

Nociceptin (Meunier et al., 1995), also known as orphanin FQ (Reinscheid et al., 1995), is the endogenous ligand for the 'orphan' opioid receptor-like 1 (ORL1) isolated from murine and human (Mollereau et al., 1994), porcine (Reinscheid et al., 1995) and rat (Bunzow et al., 1994) brain. ORL1 mRNA transcripts are expressed in the mouse and rat central nervous system (Mollereau et al., 1994; Bunzow et al., 1994), particularly in the central gray and dorsal horn of the spinal cord, as well as in peripheral organs such as intestine, vas deferens, liver and spleen (Wang et al., 1994). Nociceptin is a 17 amino acid peptide that shares structural homology (50–60%) with the endogenous opioid peptides like enkephalins, dynorphin A or β-endorphins, suggesting that its receptor may show similar properties to the opioid receptors. Like the opioid receptors, ORL1 receptor contains seven transmembrane domains and is member of G_i/G_0-coupled receptor superfamily. Nociceptin inhibits adenyl cyclase activity (Meunier et al., 1995; Reinscheid et al., 1995), Ca^{2+} entry through the voltage-dependent N-type calcium channel current in human neuroblastoma cells (Connor et al., 1996a) and in freshly dissociated hippocampal pyramidal neurons (Knoflach et al., 1996), and nociceptin mobilizes intracellular Ca^{2+} and in human neuroblastoma cell line (Connor et al., 1996a). Activation of the inwardly rectifying K^+ conductance in rat locus coeruleus (Connor et al., 1996b), dorsal raphe nucleus (Vaughan and Christie, 1996), periaqueductal gray (Vaughan et al., 1997) neurones and inhibition of K^+-evoked glutamate release in rat cerebrocortical slices

* Corresponding author. Tel.: +1-908-740-7238; fax: +1-908-740-7175.
E-mail address: michel.corboz@spcorp.com (M.R. Corboz).

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(Nicol et al., 1996) have been obtained with nociceptin. However, although the ORL1 receptor displays sequence similarity to the opioid receptors, nociceptin itself has low or no affinity for opioid receptor ligands (Mogil et al., 1996). Indeed, nociceptin does not possess the N-terminal tyrosine residue that is essential for activity at the μ-, δ- and κ-opioid receptors (Meunier et al., 1995; Reinscheid et al., 1995) but has the N-terminal phenylalanine (Mogil et al., 1996; Nicholson et al., 1998).

ORL1 receptors are expressed in mammalian central nervous system and many biological actions have been described including hyperalgesia in the brain (Meunier et al., 1995; Reinscheid et al., 1995), spinal analgesia (Stanfa et al., 1996; Xu et al., 1996), motor impairment (Reinscheid et al., 1995), suppression of spatial learning (Sandin et al., 1997) and stimulation of food intake (Pomonis et al., 1996). These receptors are also found in several peripheral organs such as airways (Patel et al., 1997; Fisher et al., 1998; Rizzi et al., 1998), left atrium (Giuliani and Maggi, 1997), kidney (Giuliani and Maggi, 1996; Bigoni et al., 1999), vas deferens (Berzetei-Gurske et al., 1996; Calo et al., 1996; Zhang et al., 1997; Nicholson et al., 1998; Bigoni et al., 1999), colon (Rizzi et al., 1999), ileum (Calo et al., 1997; Zhang et al., 1997; Nicholson et al., 1998; Bigoni et al., 1999), and nociceptin exerts a modulatory influence on transmitter release in these peripheral preparations. Nociceptin has been shown to inhibit electrically-evoked release of (1) tachykinins from sensory nerves in the guinea pig renal pelvis (Guiliani and Maggi, 1996), isolated bronchus (Fisher et al., 1998; Shah et al., 1998) and rat trachea (Helyes et al., 1997), (2) calcitonin-gene related peptide from guinea pig left atrium (Giuliani and Maggi, 1997) and rat trachea (Helyes et al., 1997), (3) acetylcholine from guinea pig isolated trachea (Patel et al., 1997) and (4) norepinephrine from rat tail arteries (Bucher, 1998).

The release of neuropeptides from sensory nerve terminals can be influenced by a variety of receptors in guinea pig lung including opioids (Frossard and Barnes, 1987; Belvisi et al., 1988; Matran et al., 1989). Because the ORL1 receptor shares significant sequence homology and multiple responses at the cellular level with the opioid receptors, and endogenous nociceptin has been localized to sensory nerve fibers within the bronchus (Fisher et al., 1998), we presently investigated whether the ORL1 receptor agonist nociceptin/orphanin FQ is able to reduce the capsaicin-induced bronchoconstriction in isolated guinea pig lung. Capsaicin causes bronchoconstriction through the release of tachykinin from afferent nerves (Lundberg et al., 1985). In addition, we also evaluated the pharmacological effect of a new ORL1 receptor antagonist 1-[(3*R*,4*R*)-1-Cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2*H*-benzimidazol-2-one (J-113397) (Kawamoto et al., 1999) in the isolated lung preparation. We also confirmed its antagonist activity on ORL1 receptor expressed in Chinese hamster ovary (CHO) cell membranes.

2. Materials and methods

2.1. Binding assays

ORL1 binding assay was performed on CHO cell membranes expressing human ORL1 receptor as described by Fawzi et al. (1997). Briefly, assays were performed using 4 μg of CHO cell membranes and 25 pM [^{125}I][Tyr^{14}]nociceptin/orphanin FQ in a 200 μl final reaction volume of buffer containing 50 mM HEPES (pH 7.4), 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM NaCl, 0.025% Bacitracin (Sigma, St. Louis, MO, USA), and 0.1% bovine serum albumin (Buffer A). Assays were carried out for 1 h at room temperature (22 °C) and were terminated by rapid filtration over GF/B membranes (Unifilter-96, Packard, Downers Grove, IL, USA) presoaked in 0.3% polyethyleneimine (Sigma). Membranes were washed five times with 1 ml cold water (4°C) and radioactivity retained on filters was determined in a Packard Top-Count microplate scintillation counter after drying of filters and addition of 50 μl of Microscint 0 (Packard). All assays were performed in duplicates. Total binding in the absence of compound and non-specific binding in the presence of 10 nM nociceptin/orphanin FQ were determined in quadruplicates. The ORL1 receptor antagonist 1-[(3*R*,4*R*)-1-Cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2*H*-benzimidazol-2-one (J-113397) was tested at eight different concentrations ranging from 0.01 nM to 1 μM. IC_{50} (concentration that inhibits 50% of binding) values were determined by curve fitting the data using the program GraphPad Prism (GraphPad Software, San Diego, CA, USA) and K_i values were calculated using the formula of Cheng and Prusoff (1973). Nociceptin/orphanin FQ shows K_i value of 0.051 ± 0.010 nM ($n = 4$) in this assay.

Opioid receptor binding assays were performed on CHO cell membranes expressing human μ-, δ-, and κ-opioid receptors (Receptor Biology, Beltsville, MD, USA). Assays were carried out using 20 μg of CHO cell membranes in a 200 μl final reaction volume of buffer A (see above). [^{3}H]Diprenorphine was used at a concentration of 0.3 nM for the μ- and κ-opioid receptors and 1 nM for the δ-opioid receptor. Reactions were carried out for 1 h at room temperature (22°C) and were terminated by rapid filtration over GF/B filters (Unifilter-96, Packard) presoaked in 0.3% polyethyleneimine followed by five washes of 1 ml cold water (4°C). Radioactivity retained on filters was counted in a Packard Top-Count microplate scintillation counter after drying of filters and addition of 50 μl of Microscint 0 (Packard). The new ORL1 antagonist J-113397 (Kawamoto et al., 1999) was tested at eight different concentrations ranging from 10 nM to 100 μM. All assays were performed in duplicates. Total binding in the absence of compounds and non-specific binding in the presence of 10 μM opioid receptor antagonist naloxone were determined in quadruplicates. IC_{50} values were deter-

mined by curve fitting the data using the program GraphPad Prism. K_i values were derived from the IC_{50} values using the formula of Cheng and Prusoff (1973). Using this assay, diprenorphine shows K_i values of 0.17 ± 0.05, 0.87 ± 0.16 and 0.23 ± 0.06 nM ($n = 3$) against the μ-, δ- and κ-opioid receptors, respectively.

2.2. [^{35}S]GTPγS binding assay

[^{35}S]GTPγS binding to CHO cell membranes expressing the ORL1 receptor was performed as described by Fawzi et al. (1997). Briefly, CHO cell membranes expressing the human ORL1 receptor (20 μg) were incubated for 30 min at room temperature with 100–300 pM [^{35}S]GTPγS in a reaction mixture (200 μl) containing 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$, 1 mg/ml Bovine serum albumin (BSA), 0.25 mg/ml bacitracin, 120 mM NaCl, and 1 μM GDP. Reactions were terminated by rapid filtration over GF/B filters presoaked for 30 min in 10 mM K_2HPO_4 and washed seven times with 2 ml cold (4°C) buffer containing 20 mM Tris–HCl (pH 8.0), 20 mM $MgCl_2$, and 100 mM NaCl. Filter-bound radioactivity was quantified by scintillation counting. Non-specific binding was determined by performing the assay in the presence of 10 μM GTPγS. For the determination of the ORL1 receptor agonist and antagonist on [^{35}S]GTPγS binding, membranes were preincubated with nociceptin/orphanin FQ or J-113397 for 60 min prior to the initiation of the assay. All assays were performed in duplicates.

2.3. Isolated perfused lungs

The isolated perfused guinea pig lung was used to investigate the effect of the ORL1 receptor agonist nociceptin/orphanin FQ against bronchoconstriction elicited by capsaicin (endogenous tachykinins) and exogenous tachykinins. Guinea pigs (400–700 g) were euthanized with an intraperitoneal overdose of sodium pentobarbital. The isolated lungs were prepared and perfused as described previously (Vemulapalli et al., 1992). Briefly, a thoracotomy was rapidly performed and the lungs and the heart were removed en bloc. The trachea and pulmonary artery were rapidly cannulated and half of the heart was cut to facilitate the drainage. The lungs were then placed inside a warmed (37°C) glass chamber and suspended from a force displacement (Grass FT-03). They were mechanically ventilated with room air using a small rodent ventilator (Harvard). The respiratory rate was set at 60 strokes/min with a volume of 2.0 ml/stroke. Pulmonary inflation pressure was continuously monitored with a pressure transducer (Gould P231D) connected to a side arm of the tracheal cannula. Perfusion pressure was maintained with a peristaltic pump (Cole-Palmer 7553-20) at a rate of 4.5–5.0 ml/min to produce a baseline pulmonary artery pressure between 6 and 14 cm H_2O. The pulmonary artery pressure was continuously monitored using a pressure transducer (Stathum P23XL) connected to the side arm of the pulmonary artery cannula. Transducers were connected to a polygraph (Grass Model #7) for continuous monitoring of variables. The lungs were perfused with a Tyrode's solution maintained at 37°C. The Tyrode's perfusate was composed of NaCl 137.0 mM, KCl 2.7 mM, $CaCl_2$ 0.4 mM, $MgCl_2$ $6H_2O$ 1.0 mM, $NaHCO_3$ 11.9 mM, NaH_2PO_4 0.4 mM and dextrose 5.5 mM.

2.3.1. Experimental protocol

One hundred and six guinea pigs were used in this study. Cumulative dose–response curves were constructed by adding capsaicin (10^{-2}–10^{6} μg/100 μl) or the tachykinin NK_2 receptor agonist neurokinin A (0.01–30 μg/ml) directly into the pulmonary artery. Capsaicin or neurokinin A were administered in increasing doses as a bolus at 2 min intervals and the volume of injectate was 0.1 ml for capsaicin and 1 ml for neurokinin A. Nociceptin/orphanin FQ (0.001–1 μM) was perfused for 30 min before the addition of increasing concentrations of capsaicin or neurokinin A. ORL1 receptor antagonists, [Phe^{1}ψ(CH_2-NH)Gly^2]nociceptin(1–13)NH_2 at 3 μM and J-113397 at 0.3 μM, and the non-selective opioid receptor antagonist naloxone at 1 μM were perfused for 5 min before the addition of nociceptin/orphanin FQ. Only one dose–response curve was obtained from each lung. Pulmonary inflation pressure, pulmonary artery pressure and tissue weight were monitored and wet/dry weight ratios were obtained from lungs dried for 12 h at 60°C.

Time-control experiments were performed in three lungs to ensure that time has no effect on pulmonary inflation pressure, pulmonary artery pressure and wet/dry weight ratios. These time-control experiments were identical in time duration to the other treatment groups, except drugs were not added to the perfusion.

One additional group (four lungs) received only the nociceptin/orphanin FQ vehicle, Dimethyl sulfoxide (DMSO) before capsaicin treatment to ensure that DMSO has no effect on capsaicin-induced bronchoconstriction. The last group (four lungs) was not treated with capsaicin and received only the capsaicin vehicle ethanol to ensure that ethanol has no effect on pulmonary inflation pressure, pulmonary artery pressure and wet/dry weight ratios.

2.3.2. Data analysis

For the construction of the dose–response curves, responses were expressed as a percentage of the maximal response to capsaicin:

$$\text{Response} = \left[(PIP_X - PIP_{BL})/(PIP_M - PIP_{BL})\right] \times 100$$

where PIP_X is the pulmonary inflation pressure measured after drug treatment, PIP_{BL} is the pulmonary inflation pressure measured before capsaicin treatment (base line) and PIP_M is the pulmonary inflation pressure measured after maximal bronchoconstriction.

An analysis of variance (ANOVA) was performed on the different treatment groups to determine significant

effects of the treatments. Post-hoc analysis between the different groups was performed with a one-sided Dunnett's *t*-test. A value of $P < 0.05$ was accepted as the level of statistical significance. All results are expressed as means ± standard error of the mean (S.E.M.).

2.4. Solution and drugs

Capsaicin was purchased from Sigma (St. Louis, MO, USA), the ORL1 receptor agonist nociceptin/orphanin FQ from Phoenix Pharmaceuticals (Mountain View, CA, USA), the ORL1 receptor antagonist [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ from Tocris Cookson (Bristol, UK), the opioid receptor antagonist naloxone from RBI (Natick, MA, USA) and the tachykinin NK$_2$ receptor agonist neurokinin A from Peninsula Labs (Belmont, CA, USA). The ORL1 receptor antagonist J-113397 was synthesized at Schering-Plough Research Institute (Kenilworth, NJ, USA). Agonists were dissolved in Tyrode's and antagonists were dissolved in DMSO then diluted in Tyrode's. Stock solution of capsaicin was made in 95% ethanol, divided into aliquots and stored in 1 ml plastic tubes. The aliquots were stored frozen and thawed the day of an experiment. Stock solutions of the other drugs were prepared daily and dissolved in Tyrode's. In binding studies, [^{125}I][Tyr^{14}]nociceptin/orphanin FQ (2200 Ci/mmol) was obtained from Amersham-Pharmacia Biotech (Cardiff, UK), [^{3}H]Diprenorphine (58 Ci/mmol) from New England Nuclear (Boston, MA, USA) and nociceptin/orphanin FQ from Bachem (Torrance, CA, USA).

3. Results

3.1. Binding studies

J-113397, ORL1 receptor antagonist inhibited [^{125}I][Tyr^{14}]nociceptin/orphanin FQ binding to the ORL1 receptor with a K_i of 2.0 nM (Table 1). This compound showed 23,104-, 418- and 89-fold selectivity for the ORL1 over the human δ-, κ- and μ-opioid receptors, respectively (Table 1). Functional characteristics of J-113397 against the ORL1 receptor was evaluated using [^{35}S]GTPγS binding assay in CHO membranes expressing the human ORL1 receptor (Fawzi et al., 1997). J-113397 shifted the concentration–response curve of nociceptin/orphanin FQ to the right (Fig. 1). Schild regression analysis of the data revealed a competitive interaction between J-113397 and nociceptin/orphanin FQ with a pK_B of 9.1.

Table 1
Effect of the ORL1 antagonist J-113397 on ORL1 and opioid receptors

Receptor	K_i (nM)	Selectivity ratio
ORL1	2.0 ± 0.2	1
δ-opioid	46208 ± 3845	23,104
κ-opioid	835 ± 162	418
μ-opioid	177 ± 4	89

K_i values were obtained using binding assays described under Materials and methods, and values shown are means ± S.E.M. ($n = 4$–5).

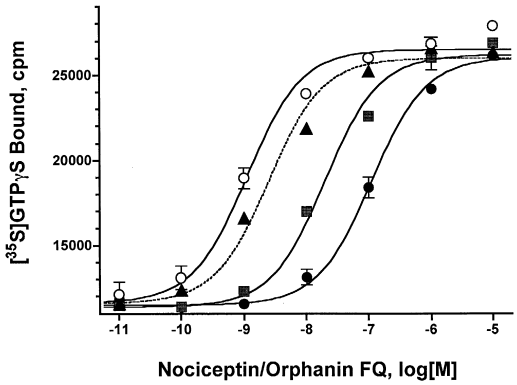

Fig. 1. Inhibition of nociceptin/orphanin FQ-stimulated GTPγS binding by the ORL1 antagonist J-113397. Nociceptin/orphanin FQ-stimulated [^{35}S]GTPγS binding to CHO cell membranes expressing the ORL1 receptor was performed as described under Materials and methods. J-113397 at 0.001 μM (closed triangles), at 0.01 μM (closed squares) and 0.1 μM (closed circles) shifted the concentration-dependent effect curve of nociceptin/orphanin FQ (open circles) to the right.

3.2. Isolated lung

3.2.1. Control experiments

In the absence of drugs, the pulmonary inflation pressure, pulmonary artery pressure and wet/dry weight ratios of isolated lungs ($n = 3$) were stable and did not differ appreciably from the beginning to the end of the experimental time frame (data not shown), indicating that the basal tone was unaffected during the course of the experiment.

When isolated lungs ($n = 4$) were treated with the capsaicin vehicle ethanol only, no significant change in pulmonary inflation pressure, pulmonary artery pressure and wet/dry weight ratios were observed (data not shown), indicating that the concentration of ethanol had no effect on the basal tone of this preparation.

The effect of the ORL1 receptor antagonist vehicle DMSO was evaluated in four isolated lungs. The EC$_{50}$ (concentration that produced 50% of the maximal response) for capsaicin-induced bronchoconstriction of lungs pretreated with DMSO only (0.30 ± 0.25 μg) was not significantly different from the EC$_{50}$ of lungs not pretreated with DMSO (0.39 ± 0.14 μg), indicating that DMSO at the concentration used in this study has no significant effect on capsaicin-induced bronchoconstriction.

3.2.2. Effect of nociceptin / orphanin FQ on capsaicin-induced bronchoconstriction in isolated lung

Capsaicin produced a dose-dependent bronchoconstriction in our preparation of guinea pig isolated lung. The

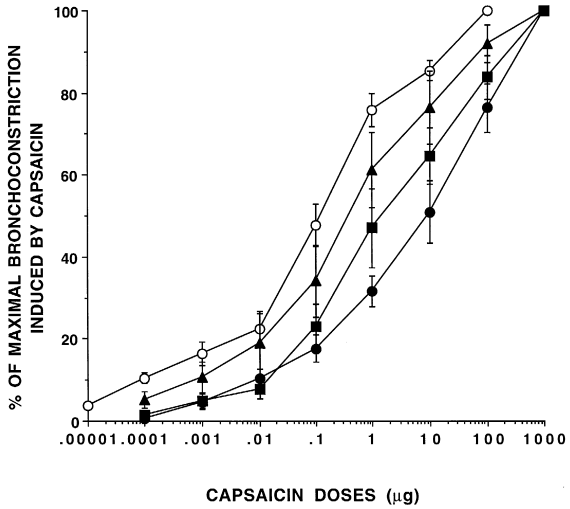

Fig. 2. Concentration-dependent inhibition by nociceptin/orphanin FQ of the capsaicin-induced bronchoconstriction in isolated guinea pig lung. Bronchoconstrictor responses are shown for control lungs (open circles, $n = 21$) and lungs treated with nociceptin/orphanin FQ at 0.001 μM (closed triangles, $n = 6$), 0.01 μM (closed squares, $n = 7$), and 1 μM (closed circles, $n = 6$). Values are mean ± S.E.M. The EC_{50} for capsaicin-induced bronchoconstriction were 0.39 ± 0.14 μg in the absence of nociceptin/orphanin FQ, 2.40 ± 1.88, 5.61 ± 2.81 and 17.00 ± 8.11 μg in the presence of nociceptin/orphanin FQ at 0.001, 0.01 and 1 μM, respectively, and the maximal changes ($PIP_M - PIP_{BL}$) in pulmonary inflation pressure (PIP) were 36.1 ± 1.5, 37.9 ± 3.1, 36.1 ± 3.5 and 36.6 ± 3.5 mm Hg, respectively.

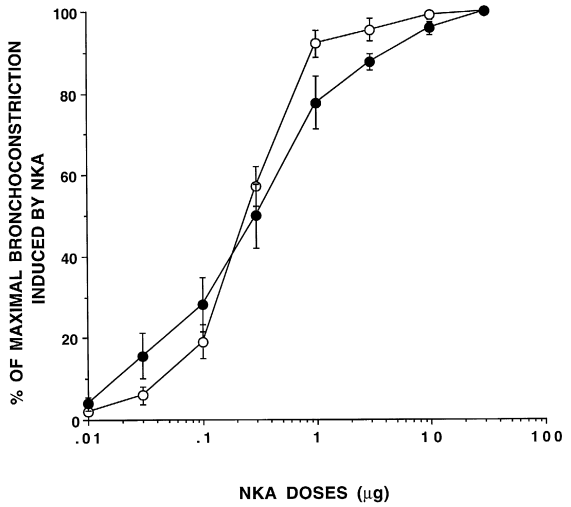

Fig. 3. Effect of nociceptin/orphanin FQ on neurokinin A (NKA)-induced bronchoconstriction in isolated guinea pig lungs. Bronchoconstrictor responses are shown for control lungs (open circles, $n = 6$) and lungs treated with nociceptin/orphanin FQ at 1 μM (closed circles, $n = 7$). The maximal changes ($PIP_M - PIP_{BL}$) in pulmonary inflation pressure (PIP) were 52.3 ± 2.8 and 48.1 ± 2.5 mm Hg, respectively. Note that nociceptin/orphanin FQ had no effect on neurokinin A-induced bronchoconstriction. Values are means ± S.E.M.

averaged pulmonary artery pressure (8.2 ± 0.3 mm Hg) and weight (8.9 ± 0.6 g) after the first dose of capsaicin (0.00001 μg) were not significant different (ANOVA) from the averaged pulmonary artery pressure (8.6 ± 0.3 mm Hg) and weight (9.8 ± 0.6 g) after the highest dose of capsaicin (100 μg). This observation suggests that the response to capsaicin is not related to lung edema. Nociceptin/orphanin FQ caused a concentration-dependent inhibition of the capsaicin-induced bronchoconstriction (Fig. 2). The EC_{50} for capsaicin-induced bronchoconstriction was significantly greater in presence of 0.01 μM nociceptin/orphanin FQ (5.61 ± 2.81 μg) ($n = 7$) than in its absence (0.39 ± 0.14 μg) ($n = 21$). The peptidic ORL1 receptor antagonist [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin(1–13)$NH_2$ at 3 μM (EC_{50} of 0.80 ± 0.33 μg) ($n = 6$) and the nonpeptidic ORL1 receptor antagonist J-113397 at 0.3 μM (EC_{50} of 0.39 ± 0.21 μg) ($n = 7$) were able to reverse the inhibitory effects of 0.01 μM nociceptin/orphanin FQ, whereas the opioid receptor antagonist naloxone at 1 μM (EC_{50} of 4.85 ± 1.41 μg) ($n = 7$) had no effect on capsaicin-induced bronchoconstriction (Table 2). The ORL1 receptor antagonists alone and naloxone alone had no effect on capsaicin-induced bronchoconstriction (EC_{50} for [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin(1–13)$NH_2$ at 3 μM ($n = 7$), J-113397 at 3 μM ($n = 6$) and naloxone at 1 μM ($n = 8$) were 0.42 ± 0.24, 0.13 ± 0.09 and 1.28 ± 0.82 μg, respectively).

To assess whether the effect of nociceptin/orphanin FQ may have a post-junctional component, we examined its effect on the exogenous neurokinin A-induced bronchoconstriction. Neurokinin A produced a dose-dependent bronchoconstriction ($n = 6$) without significantly affecting pulmonary arterial pressure and wet/dry ratios. Addition of nociceptin/orphanin FQ (1 μM) did not affect the neurokinin A-induced bronchoconstriction ($n = 7$) (Fig. 3). We have previously demonstrated that neurokinin A-in-

Table 2
EC_{50}s and dose ratios for various treatments

Compound	Dose (μM)	*n*	EC_{50} (μg)	Dose ratio
Capsaicin	–	21	0.39 ± 0.14	–
Nociceptin/orphanin FQ	0.01	7	5.61 ± 2.81[a]	14.4
Nociceptin/orphanin FQ + [F/G]NC(1–13) NH_2	0.01 + 3	6	0.80 ± 0.33	2.1
Nociceptin/orphanin FQ + J-113397	0.01 + 0.3	7	0.39 ± 0.21	1.0
Nociceptin/orphanin FQ + naloxone	0.01 + 1	7	4.85 ± 1.41[a]	12.4

Dose ratio is treated EC_{50}/control EC_{50}.

[a] Indicates significant difference ($P < 0.05$) from capsaicin, nociceptin/orphanin FQ (0.01 μM) + [$Phe^1\psi(CH_2\text{-}NH)Gly^2$]nociceptin (1–13)$NH_2$) ([F/G]NC(1–13) NH_2) (3 μM), and nociceptin/orphanin FQ (0.01 μM) + J-113397 (3 μM) groups.

duced bronchoconstriction was significantly inhibited by the tachykinin NK2 receptor antagonist SR 48968 (Rivelli et al., 1999). The EC_{50} for neurokinin A-induced bronchoconstriction were not significantly different between the presence (7.6 ± 1.3 ng) ($n = 6$) and the absence (25.3 ± 16.1 ng) ($n = 7$) of nociceptin/orphanin FQ.

4. Discussion

The present study shows for the first time that ORL1 receptor agonist nociceptin/orphanin FQ inhibited capsaicin-induced bronchoconstriction in the isolated guinea pig lung, a response mediated by the release of endogenous tachykinins from sensory nerves. This inhibitory effect of nociceptin/orphanin FQ was selectively blocked by the ORL1 receptor antagonists, [Phe$^1\psi$(CH_2-NH)Gly^2]nociceptin(1–13)NH_2 and J-113397, whereas the non-selective opioid antagonist naloxone had no effect. Nociceptin/orphanin FQ did not affect the bronchoconstriction induced by exogenously applied tachykinin NK_2 receptor agonist neurokinin A indicating that the inhibitory activity of nociceptin/orphanin FQ was exerted at the prejunctional level on sensory nerve terminals. Both the agonist activity of nociceptin/orphanin FQ and the antagonist activity of J-113397 were also confirmed on CHO cell membranes. Schild analysis of the antagonist activity of J-113397 at 3 μM revealed a competitive type of antagonism with a pK_B of 9.1.

Nociceptin/orphanin FQ is a recently discovered opioid-like peptide acting on ORL1 receptors and sharing many similarities to the opioid peptide family at both the cellular level and amino acid sequence (Mollereau et al., 1994; Meunier et al., 1995; Reinscheid et al., 1995). The ORL1 receptor type is present in peripheral organs of several species and mediates inhibitory effects in different nerve terminals. Nociceptin/orphanin FQ inhibits the depolarization-evoked transmitter release from (1) cholinergic nerves in the guinea pig ileum (Calo et al., 1997; Zhang et al., 1997; Nicholson et al., 1998; Bigoni et al., 1999) and trachea (Patel et al., 1997), (2) adrenergic nerves in the mouse (Berzetei-Gurske et al., 1996; Calo et al., 1997; Zhang et al., 1997; Bigoni et al., 1999), rat (Nicholson et al., 1998; Bigoni et al., 1999) and rabbit (Nicholson et al., 1998) vas deferens and rat tail artery (Bucher, 1998), and (3) sensory nerves in the guinea pig renal pelvis (Giuliani and Maggi, 1996; Bigoni et al., 1999), bronchus (Fisher et al., 1998; Rizzi et al., 1998), left atrium (Giuliani and Maggi, 1997) and rat trachea (Helyes et al., 1997). Therefore, ORL1 receptors are found in sympathetic, parasympathetic and sensory nerves, an observation similar to the distribution of opioid receptors. But the inhibitory effect of nociceptin in all these studies was not affected by opioid receptor antagonist indicating that nociceptin is not acting through an opioid receptor. In the present study in isolated guinea pig lung, nociceptin/orphanin FQ inhibited capsaicin-evoked bronchoconstriction in a concentration dependent manner, suggesting a neuromodulatory effect of nociceptin/orphanin FQ on capsaicin-induced release of neurokinins from capsaicin-sensitive sensory nerve endings. To date, the inhibitory action of nociceptin on transmitter release from guinea pig airways has been described only in in vitro isolated bronchus (Patel et al., 1997; Fisher et al., 1998; Shah et al., 1998; Rizzi et al., 1998). It is interesting to note that in guinea pig isolated bronchus, nociceptin has no effect on the contractions elicited by capsaicin (Fisher et al., 1998; Shah et al., 1998), a result in conflict with the present study. This observation could depend on the experimental difference between the two preparations, isolated bronchus and isolated lungs. The incubation time of ORL1 receptor agonist was 30 min in our preparation, 10 min (Shah et al., 1998) and 15 min (Fisher et al., 1998) in the studies of guinea pig isolated bronchus. Moreover, nociceptin/orphanin FQ was directly injected in the pulmonary artery in the preparation of isolated lungs, whereas nociceptin was added to the tissue chamber in isolated bronchus preparation, and the diffusion of the drug is likely to be very different in these two preparations. Also, drug injected through the blood vessel in isolated lung preparation may cause vascular extravasation and this could contribute to the bronchoconstriction. Thus, extrapolation from one technique to a more sophisticated and complex model requires caution. In support of this, adrenomedullin, a peptide isolated from human pheochromocytoma tissue, inhibits acetylcholine-induced bronchoconstriction in in vivo guinea pigs (Kanasawa et al., 1994) whereas it did not alter acetylcholine-induced bronchoconstriction in tracheal rings and bronchial strips (Pinto et al., 1996). Also, in isolated perfused murine lung, serotonin and methacholine were equally effective as bronchoconstrictor agents (Held et al., 1999) whereas the maximum response in isolated tracheal rings to serotonin was only 25% of that of methacholine with EC_{50} values of 0.4 μM for serotonin and 5000 μM for methacholine, respectively (Garssen et al., 1990). Another example is the endotoxin-induced release of prostaglandins that occurs in rat pulmonary tissue in vivo (Feuerstein and Ramwell, 1981) and perfused lung (Uhlig et al., 1996), but not in lung strips (Feuerstein and Ramwell, 1981).

We also found that the nociceptin/orphanin FQ-induced inhibition of the capsaicin effect was antagonized by the nonpeptidic ORL1 receptor antagonist recently identified by J-113397 (Kawamoto et al., 1999). In our preparation of isolated guinea pig lung, this compound showed a better dose ratio (1.0) than the standard ORL1 receptor antagonist [Phe$^1\psi$(CH_2-NH)Gly^2]nociceptin(1–13)NH_2 (2.1). [Phe$^1\psi$(CH_2-NH)Gly^2]nociceptin(1–13)NH_2 was the first peptide ORL1 receptor antagonist that acts as a competitive antagonist with pA_2 values of 7.02 and 6.75 in the guinea pig ileum and mouse vas deferens, respec-

tively (Guerrini et al., 1998). However, partial or even full agonistic activity of [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin(1–13)NH$_2$ has been reported in rat and mouse colon (Corbett et al., 1998; Rizzi et al., 1999). Also, two δ-opioid receptor ligands carbetapentane and rimcazole, and a derivative compound of the μ-opioid receptor antagonist naloxone, naloxone benzoylhydrazone have been shown to act as ORL1 receptor antagonists (Kobayashi et al., 1997, Noda et al., 1998), but these compounds also interact with μ-, δ- and κ-opioid receptors and with muscarinic M$_1$ receptors for carbetapentane (Kobayashi et al., 1997; Kawamoto et al., 1999) and therefore are of little utility for receptor characterization. Conversely, the non-selective opioid antagonist naloxone (1 μM) was completely ineffective in our isolated lung preparation, indicating that nociceptin/orphanin FQ lacks activity at the μ-, δ- and κ-opioid receptors. A concentration of 0.3 μM naloxone (Shah et al., 1998) and 1 μM naloxone (Rizzi et al., 1998) were able to block the effects of opioid receptor agonists without modifying the inhibitory effects of nociceptin in guinea pig isolated bronchus, suggesting that the dose used in the present study (1 μM) was the appropriate dose. Presently, we did not investigate which mechanism is involved in the nociceptin/orphanin FQ-induced inhibition of the capsaicin effect, but one of the processes involved could depend on the activation of large conductance K^+ channels. Opioid receptor agonists inhibit prejunctional tachykinin release by a mechanism involving the potassium channels (Stretton et al., 1992).

Capsaicin induces the release of endogenous tachykinins from lung sensory nerve endings (Lundberg et al., 1985) leading to bronchoconstriction. Substance P, neurokinin A and calcitonin gene-related peptide are the neuropeptides released by capsaicin from central and peripheral endings of afferent neurons (Holzer, 1988) and exert a number of effects directly on smooth muscle tissues. We have previously shown in the same preparation that capsaicin-induced bronchoconstriction is tachykinergic in nature (Tozzi et al., 1996). Indeed, the bronchoconstriction was inhibited by the tachykinin NK$_2$ receptor antagonist SR 48968 whereas the tachykinin NK$_1$ receptor antagonist CP 99994 had no effect (Tozzi et al., 1996) indicating that tachykinin NK$_2$ receptors are predominantly involved. We also demonstrated in the same preparation that the exogenously applied tachykinin NK$_2$ agonist receptor neurokinin A produced a dose-dependent bronchoconstriction (0.01–30 μg/ml) by acting directly on smooth muscle tachykinin NK$_2$ receptors (Rivelli et al., 1999). SR 48968 (0.03–0.3 μM) produced dose related inhibition of neurokinin A-induced bronchoconstriction and CP 99994 did not affect it (Rivelli et al., 1999). In the present study, nociceptin/orphanin FQ inhibited the bronchoconstriction induced by capsaicin without affecting that of exogenously administered neurokinin A indicating a prejunctional site of action on tachykinin-containing sensory nerve terminals. In support of this mechanism, nociceptin is able to produce a prejunctional inhibition of neurotransmitter release in different preparations, such as in guinea pig renal pelvis (Giuliani and Maggi, 1996) and isolated bronchus (Rizzi et al., 1998).

In conclusion, our results demonstrated in isolated guinea pig lung that (1) nociceptin/orphanin FQ inhibits capsaicin-induced bronchoconstriction, (2) this effect is mediated via the activation of ORL1 receptors because the inhibitory effect is antagonized by the ORL1 receptor antagonists 1-[(3*R*, 4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397) and [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin (1–13)NH$_2$, whereas the non-selective opioid receptor antagonist naloxone has no effect, and (3) nociceptin/orphanin FQ can modulate neuroeffector transmission through an inhibitory prejunctional mechanism on capsaicin-evoked tachykinin release from capsaicin-sensory nerve terminals. These findings suggest a possible use of a nociceptin receptor agonist in treatment of respiratory disorders that have a neurogenic component.

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